REMARKS

Claims 1-63 are pending in the application. Claims 1, 35, and 62-63 have been amended. Claims 2-3 and 36-37 are cancelled without prejudice. Support for the amendments to claims can be found throughout the specification, for example in claims 3 and 37 of the application as filed. After entry of the amendment, claims 1, 4-35, and 38-63 will be pending.

Objection to the Specification:

The specification has been objected to for the status of the parent application not being up to date. Applicants have amended the specification as set forth above to include the patent number of the issued patent. The amendment does not constitute new matter. Therefore, the objection is overcome.

Rejections under 35 U.S.C. §102(b):

The Examiner has rejected claims 1, 11-14, 16, 22, 23, 25, 62, and 63 under 35 U.S.C. 102(b) as being anticipated by Wiesner (Nuc. Acid Res. 1992).

Applicants have amended claim 1 to include the limitation of claim 3 which is not included in the rejection. Therefore, the newly amended claim 1 is not anticipated by Wiesner. As claims 11-14, 16, 22, 23, and 25 are dependent on the now non-anticipated claim 1, they are also non-anticipated.

Claims 62 and 63 have been amended to include the limitation of separating and detecting at least 5 nucleic acid species in the aliquot analyzed. Per the statement of the Examiner in the 103(a) rejection on page 3 of the office action, "Wiesner does not disclose use of at least three different amplification templates." Therefore, the newly amended claims 62 and 63 cannot be anticipated by Wiesner.

Therefore, the rejection of the claims for anticipation over Wiesner is overcome.

Applicants respectfully request reconsideration and withdrawal of the §102 rejection over this reference.

Rejections under 35 U.S.C. §103:

The Examiner has rejected claims 2-10, 15, 17-21, 24, and 26-34 under U.S.C. 103(a) as being unpatentable over Wiesner in view of Schumm (USP 6,479,235).

The Examiner states that:

> One of ordinary skill in the art would have been motivated to use a plurality of amplification targets, such as more than three, as well as the technology of capillary electrophoresis, in the method of Wiesner, because of the advantages of both multiplex amplification and capillary electrophoresis were well known and common knowledge in the art, as demonstrated by Schumm et al. In other words, the skilled artisan considering these references would have been motivated to apply multiplex amplification and capillary electrophoresis as taught by Schumm et al. in the method of Wiesner to provide the obvious advantage of facilitating quantitative amplification profiles of large numbers of target nucleic acids. It would have been prima facie obvious to one of ordinary skill in the art at the time of the invention to carry out the claimed methods.

Applicants respectfully disagree.

Applicants submit that the quantitative method of Wiesner relies on high efficiency reverse transcription and short cDNAs. The second to last paragraph of the reference states:

> However, the method presented here measures true mRNA molecule numbers only if all mRNA templates are reverse transcribed into cDNA. Amplification for 12 cycles of samples taken from the reverse transcription reaction at different time points yielded the same incorporation of radioactivity from 15 minutes up to 1 hour (data not shown). In addition, decreasing the concentration of the substrate RNA form 1 μg down to 30 ng by two-fold dilution steps yielded the same incorporation of radioactivity after amplification, however, one cycle later for each dilution step (data not shown). Thus, cDNA synthesis is completed under these conditions and does not depend on the primer to template ratio, as would have been expected if the efficiency of reverse transcription were less than 100%. Moreover, Berger *et al.*, (10) have shown that 50% of input mRNA was reverse transcribed into full-length cDNA under comparable conditions, but using only a 2-3 fold excess of primer over template. Since primers were present in our standard assay in at least 50,000-fold excess, and the cDNAs which are generated are less than 100 bp long, we conclude that all template mRNAs were reverse transcribed and that the method presented herein is indeed a quantitative one.

Applicants submit that due to the short cDNAs required for the quantitative method of Wiesner, it would be difficult, at best, to determine the quantity of a plurality of separated nucleic acid species in an aliquot, wherein a plurality of different amplification templates comprises at least five different amplification templates. The difficulty would be due to both the narrow range of permissible sizes of amplification products that could be generated within the strict limitations of

Wiesner, and the possibility of the loss of the quantitative generation of amplification products due to the high concentration of a large number of primers as would be required to modify Wiesner to a multiplex reaction.

Schumm provides "materials and methods ... for use in simultaneously amplifying at least 13 loci of genomic DNA in a single multiplex reaction." (abstract) The materials include a number of oligonucleotide primers provided in the sequence listing for use in the amplification reactions. The primers range in length from 19 to 33 nucleotides in length. It is estimated that the average primer length used in the method of Schumm is about 25 nucleotides in length. Such a length would be required to insure specificity of the primers in the multiplex reaction.

As stated above, the method of Wiesner requires the use of a very high concentration of primers (2.5 μM each, which is about 20-fold higher than the 0.12 μM used by Schumm (col 19, ln 17), and that the same primers are used for the reverse transcription and amplification reactions. In order to amplify at least 5 amplification templates, at least 10 primers would need to be included in the reverse transcription and amplification reactions. As the primers would be present in a substantial excess relative to the template, especially in early cycles; therefore, primer dimer formation could be favored over annealing to template. This would remove primer from the available pool, potentially decreasing the quantitative aspect of the method that is essential to Wiesner. Primer dimer formation could interfere with the detection of any products of about 50 nucleotides in length (about twice the length of a primer) at the end of the amplification reaction. Moreover, with the high concentration of 10 primers, it is possible that higher order structures of more than 2 primers could be generated. It is noted that all of the amplification products detected by Schumm appear to be at least about 100 nucleotides in length.

In addition to a high concentration of primers, Wiesner requires the use of cDNAs that are less than 100 nucleotides in length. In order to have quantitative amplification as required by Wiesner, the primers would need to be sufficiently closely spaced so that essentially all of the cDNAs generated in the reverse transcription reaction would be able to bind the second primer for amplification. Therefore, at most, the size of a final amplification product could be about 90 nucleotides in length to insure quantitative amplification.

Therefore, in order to modify the method of Wiesner to allow for detection of at least 5 amplification templates, primers would need to be designed to result in amplification products

that were greater than about 50 nucleotides in length, and less than about 90 nucleotides in length. Moreover, a number of control reactions would need to be run to insure that there is no cross reaction between the primers to result in the formation of unintended products within the 50 to 90 nucleotide range. Further, the primers would need to be designed such that all of the primers had about the same annealing/melting temperatures so that reverse transcription and amplification of the targets occurred in a quantitative manner.

Applicants submit that given at least the reasons provided above, one would not be motivated to modify the method of Wiesner, with its strict limitations required to make the method quantitative, to create a method to allow for multiplex detection of at least 5 amplification templates in a single reaction mixture. Applicants submit that the process of primer design would be so onerous and limiting, that it would be simpler to run multiple samples. The goal of Wiesner is to provide a method that bypasses a laborious control, *in vitro* synthesis of an RNA standard (see last paragraph of the reference), which is typically required for quantitative RT-PCR. One would not be motivated to modify Wiesner to simply exchange one laborious control for another.

> "There are three possible sources for a motivation to combine references: the nature of the problem to be solved, the teachings of the prior art, and the knowledge of persons of ordinary skill in the art." *In re Rouffet*, 149 F.3d 1350, 1357, 47 USPQ2d 1453, 1457-58 (Fed. Cir. 1998) The mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination. *In re Mills*, 916 F.2d 680, 16 USPQ2d 1430 (Fed. Cir. 1990)

Applicants submit that the multiplex methods of Schumm can only be incorporated into the method of Wiesner with substantial difficulty. As the goal of Wiesner is to eliminate complex controls, the combination of references would be improper. It is improper to combine references where the references teach away from their combination (*In re Grasselli*, 713, F.2d 731, 743, 218 USPQ 769, 779 (Fed. Cir. 1983). Moreover, it is possible that the combination of the methods would alter the quantitative nature of the Wiesner method due to template independent primer interactions. Therefore, the combination could render the prior art unsatisfactory for its intended purpose (*In re Gordon*, 733 F.2d 900,221 USPQ 1125 (Fed. Cir. 1984).

Applicants further submit that one would not be motivated to modify Schumm to incorporate the quantitative methods of Wiesner. Schumm is concerned with the amplification of at least 13 polymorphic loci known to be present in genomic DNA. Schumm is not concerned with the quantity of the amplification product, but instead with the size of the amplification product. The quantitation of the amount of product produced is neither required nor desired as it would only add unnecessary complexity to the method. Therefore, there would be no motivation to combine the methods.

Applicants respectfully request reconsideration and withdrawal of the §103 rejection over this combination of references.

The Examiner has rejected claims 35-61 under U.S.C. 103(a) as being unpatentable over Wiesner in view of Brenner (USP 6,479,235), and further in view of Schumm.

The Examiner relies on the combination of Wiesner and Schumm as set forth above, and relies on Brenner to provide teachings regarding "methods of measurement of gene expression profiles from two different gene expressing entities (test vs. control organisms)."

For the reasons set forth above, Applicants submit that the combination of Wiesner and Schumm is improper. Brenner provides no teachings to reconcile the incompatibilities of the teachings of Wiesner and Schumm. Therefore, claims are not obvious in view of the references.

Applicants respectfully request reconsideration and withdrawal of the §103 rejection over this combination of references.

In view of the above, all issues raised in the Office Action have been addressed herein. Reconsideration of the claims is respectfully requested. If there are any issues outstanding that the Examiner believes could be resolved by a telephonic interview, the Examiner is encouraged to contact the undersigned Agent for Applicant listed below.

Respectfully submitted,

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